## REMARKS

### Formal Matters

Claims 1-3 and 20 are pending.

Claims 1-3 and 20 were examined and rejected.

Claims 21 and 22 are new. Support for new claims 21 and 22 are found at, for example, page 44, lines 3-11, page 46, lines 21-29, and page 56, lines 17-21.

Applicants respectfully request reconsideration of the application in view of the remarks made herein.

### Rejections under 35 U.S.C. § 103- general discussion

In this response, the Applicants address several issues raised in the Advisory Action. The Applicants note that all arguments presented in the prior response still apply with equal force. The Applicants' prior arguments are set forth in this response after the issues in the Advisory Action have been addressed. The Applicants request reconsideration of the claims, in view of the discussion that follows below, in combination with their prior arguments.

On page 3, lines 13-19 of the Office Action, the Examiner appears to construe the Applicants' prior arguments to be directed to the patentability of a retroviral vector comprising a wild type *Renilla* GFP *nucleotide* sequence. This is not the case. The Applicants prior arguments, as well as the claims, are directed to a retroviral vector comprising a polynucleotide encoding a wild type *Renilla* GFP *amino acid* sequence. Because of the degeneracy of the genetic code, such a polypeptide can be encoded by a variety of different nucleic acid sequences, including a nucleic acid sequence that is optimized for expression in mammalian cells.

On of page 3, line 19 to page 4, line 3 of the Office Action, the Examiner states that the teachings of several references that support the Applicants' position (i.e., Aran, Hanzano, Levy, and Cheng) have been considered but are not persuasive because those references are specifically drawn to retroviral vectors encoding a wild type *Aequoria* GFP, rather than a wild type *Renilla* GFP. Since the

references are drawn to retroviral vectors encoding a wild type *Aequoria* GFP and not a wild type *Renilla* GFP, they are thought by the Examiner to be not relevant to the question at hand.

The Applicants submit, however, that if the Examiner's assertion were true, i.e., that a reference drawn to a retroviral vector encoding a wild type *Aequoria* GFP simply isn't relevant to the patentability of a claim directed to a retroviral vector encoding a wild type *Renilla* GFP, then such a reference should not be citable to render obvious claims directed to that very same subject matter. In other words, this position in the Office Action appears to be inconsistent with the fact of the use of references relating to *Aequoria* GFP as the basis for the obviousness rejection. Either the teachings of these references should be given full weight in supporting the Applicants' arguments, or the rejection should be withdrawn as being based on references that are not relevant to the claimed subject matter.

In lines 10-15 of page 4 of the Office Action, the Examiner notes that the Aran successfully expressed a humanized (i.e., codon optimized), red-shifted *Aequoria* GFP and argues that because of their success, there would be sufficient motivation to produce a codon optimized wild type *Renilla* GFP. However, the particular *Aequoria* GFP referred to by the Examiner is not only codon optimized, but also "red-shifted". As explained in *Cheng* and *Levy* (as provided to the Examiner in the Applicants' prior response), a red-shifted *Aequoria* GFP is a *mutated* Aequoria GFP, not a wild-type GFP. As such, in line with the Applicants' prior arguments, Aran's success in expressing a codon optimized, red-shifted (i.e., altered) GFP would provide no motivation to express a wild type GFP. To the contrary, since Aran clearly states that that they were *unsuccessful* in expressing wild type (i.e., not red-shifted) GFP, the Applicants prior arguments still stand with full force.

Further, the Applicants note that *Hanazano* (also provided to the Examiner in the Applicants' prior response), in the fifth line of the abstract, very clearly states that they failed to express "humanized" (i.e., human codon optimized) wild type *Aequoria* GFP using a retroviral vector. According to Hanazano, wild-type *Aequoria* GFP cannot be expressed using in human cells a retroviral vector, even if the nucleic acid encoding that protein is optimized for expression in human cells. Hanazano's findings therefore support the Applicant's position, and weaken that of the Examiner.

In page 4, line 15 to page 5, line 5 of the Office Action, the Examiner appears to argue that Aran, Cheng and Levy each teach "low level" expression of wild-type *Aequoria* GFP using a retroviral vector, and, as such, there might be some motivation to express wild-type *Renilla* GFP, albeit at a low level.

In response, the Applicants submit that it would be unfairly optimistic to characterize the Aran, Levy and Chang's expression of wild type GFP from a retroviral vector as being "low level". Aran quite clearly states on the second ¶ of page 204 that that they were "unable to detect any fluorescence by FACS analysis or microscopy" and Levy shows that wild type GFP expression is *undetectable* in fibroblast and melanoma cells, and virtually undetectable in all other cells tested. Cheng, on the other hand, never actually tested for expression of wild-type *Aequoria* GFP using a retroviral vector. As such, it would be more fair to characterize the expression level of wild type GFP from a retroviral vector as described in these references as "undetectable".

Each of these references (as well as Hanazano) highlight the problems of expressing wild-type GFPs using a retroviral vector, rather than suggest the use of such a vector. As such, these references teach away from, rather than suggest, what is being claimed. At best, the references suggest that a retroviral vector expressing a wild-type GFP would be useless because GFP expression would be undetectable. This is exactly the point that the Applicants are trying to make: the art points towards a vector that doesn't work.

Finally, the Examiner notes that the expression level of the wild-type *Renilla* GFP is not recited in the rejected claims, and, as such, there is no way to distinguish that which is being claimed from what the Examiner argues is suggested by the cited references.

First, the Applicants note that the Aran states that wild-type GFP is *undetectable* and, as such, the assertion that the subject matter of the claims is suggested in the prior art references is not supported.

Further, the Applicants submit that expression of wild type *Renilla* GFP at a detectable level is an inherent property of what is being claimed and, as such, such a recitation should not be required.

Finally, and without an intention to acquiesce to this aspect of the rejection, two new claims are presented. The first claim, claim 21, is directed to a cell comprising a retroviral vector encoding wild type *Renilla* GFP, where fluorescence can be detected by FACS. Since Aran quite clearly states that they were "unable to detect any fluorescence by FACS analysis", in the second ¶ of page 204, Aran teaches away from the subject matter of claim 21.

Likewise, the second new claim, claim 22, is directed to a cell comprising a retroviral vector encoding wild type *Renilla* GFP, where the cell is in the presence of a test agent, and wherein an effect of said test agent is detectable by detecting the fluorescence of said GFP. Since Aran would predict that a cell

comprising a retroviral vector encoding wild type GFP would not provide detectable fluorescence, Aran fails to teach or suggest all of the elements of claim 22.

Further, with particular respect to claim 22, the Applicants note that screening assays in which the effects of a test agent are detected by detecting GFP expression are not disclosed in any of the cited references. As such, for a separate reason, all of the elements of claim 22 are suggested by the prior art.

In order to ensure the arguments are preserved for the record, Applicants' prior arguments are set forth below. The Applicants request reconsideration of the claims, in view of their prior arguments supplemented by the above discussion.

**Applicants prior arguments**

The rejected claims relate to a retroviral vector containing a polynucleotide encoding wild-type *Renilla* GFP. The Applicants note that the claimed vector is a ***retroviral*** vector and the *Renilla* GFP sequence is ***wild-type***. SEQ ID NO:2 is the amino acid sequence of the wild-type *Renilla* GFP.

The claims stand rejected over Bryan (disclosing a sequence of a wild type *Renilla* GFP) in view of Aran and/or Zolutukhin (each disclosing a retroviral vector encoding human codon optimized, mutated *Aequoria* GFP). According to the Office, Aran and/or Zolutukhin's retroviral vector, in combination with Brian's wild type *Renilla* GFP sequence, render the claims unpatentable.

The claimed retroviral vector may be used to express wild type *Renilla* GFP in mammalian cells. Results supporting this statement are shown in the instant specification.

Simply put, the results achieved with the claimed invention were unexpected because the Applicants found success in an area in which others had found only failure: namely expression of wild type green fluorescent proteins using a retroviral vector. The Applicants' success was surprising because the art at the time of filing shows that wild-type GFPs other than the wild type *Renilla* GFP (i.e., the wild-type versions of the particular mutant GFP used by Aran, Zolutukhin and others) could **not** be expressed in a mammalian cell using a retroviral vector.

The Applicants' position is factually supported by the publications of Aran, Hanazano, Levy, Cheng and, and others, who unsuccessfully tried to express wild-type *Aequoria* GFP using a retroviral vector.

The publications were published before the time of filing of the instant application and were readily available at the time of filing.

In support of the Applicants' position, the Examiner's attention is drawn to the first full paragraph of page 204 of Aran's disclosure. In this paragraph, Aran states that when a retroviral vector encoding a wild type *Aequorea* GFP was introduced into in a mammalian cell, fluorescence was "***undetectable***".

Several other research groups -- namely those of Hanazono, Levy, and Cheng -- independently experienced problems in this same effort.

Hanazono (Hum. Gene Ther. 1997, 8:1313-9; Exhibit A) stated in the abstract that "many attempts by our laboratory to isolate stable retroviral producer cell clones secreting biologically active vectors containing either the highly fluorescent S65T-GFP mutant or humanized GFP have ***failed***", and with reference to retroviral vectors encoding GFP, stated in the overview "***stable clones produced neither virus nor GFP***" and "GFP may not be a suitable selective marker in mammalian gene transfer systems".

Levy et al. (Nature Biotechnology 1996, 14: 610-4, at p. 613, first full paragraph; Exhibit B) states that "Our experiments are in agreement with these results in that transient transfection which transfers multiple transgene copies of wildtype GFP expression cassettes were visualized, but we found that stable transduced lines with a single transgene copy of ***wildtype GFP could never be visualized*** by fluorescence microscopy (Table 1)".

Likewise Cheng et al (Nature Biotechnology 1996, 14: 606-609; paper enclosed as Exhibit C) states in the second paragraph of the introduction "the expression and detection of wildtype GFP (wtGFP) in mammalian cells reportedly ***failed***".

Further, several other publications generally state that wildtype *Aequorea* green fluorescent proteins are toxic to living cells (see Lie et al Biochem. Biophys. Res. Commun. 1999, 260:712-717, Duisit et al. Mol. Ther. 2002 6:446-454 and various publications by Stratagene, enclosed as Exhibits D, E and F).

It was not until the publications of Levy et al (Nature Biotechnology 1996, 14: 610-4; Exhibit B) and Cheng et al (Nature Biotechnology 1996, 14: 606-609; Exhibit C) that red-shifted, humanized, optimized ***variants*** of *Aequorea* GFP suitable for use in retroviral vectors became available. Identifying these *Aequorea* GFP variants was not a trivial task.

Aran, Hanzano, Levi and Cheng clearly state their failure to express wild type *Aequoria* GFP using a retroviral vector.

The Applicants submit that in view of the known difficulties in using retroviral vectors to express the *Aequorea* wild type green fluorescent proteins, one of skill in the art would fully expect that a retroviral vector encoding a wild type *Renilla* GFP would also fail. In direct contrast to the prevailing wisdom at the time of filing, the inventors discovered that ***wild type Renilla GFP*** could, in fact, be expressed in mammalian cells using a retroviral vector. The Applicants submit that this result was unexpected, and could not have been predicted from the art at the time of filing.

During the interview, the Examiner asked the Applicants if they knew of any reason why, in view of the precedent for failure, the inventors found success in using the claimed invention.

It is the Applicants' understanding that exact mechanism by which *Aequoria* GFPs fail to be expressed using a retroviral vector is not known and, as such, the underlying reasons for the Applicants' success are not understood. Furthermore, the Examiner is reminded that it is well established that an understanding of the scientific theory or principle underlying an invention is not a requirement for patentability.[1] Thus, while the exact molecular mechanism underlying the Applicants' success may be an interesting topic for discussion, such a discussion should have no bearing on the patentability of the rejected claims.

The general discussion set forth above supports the Applicants' position that Aran, Bryan and/or Zolutukhin, cannot be combined to render the claimed invention obvious. Withdrawal of rejections that rely on the disclosures of these references is respectfully requested.

Each of the rejections set out in the Office Action is addressed in detail below.

---

[1] See, e.g., In re Gazave, 379 F.2d 973, 978, 154 USPQ 92, 96 (CCPA 1967 ); In re Chilowsky, 229 F.2d 457, 462, 108 USPQ 321, 325 (CCPA 1956) and Philip Morris, Inc. v. Brown & Williamson Tobacco Corp., 641 F. Supp. 1438, 1483 n.13, 231 USPQ 321, 355 n.13 (M.D. Ga. 1986).

**Rejection under 35 U.S.C. § 103 - Bryan and Aran**

Claims 1-3 and 20 remain rejected under 35 U.S.C. § 103(a) as being unpatentable over Bryan and Aran. The Office Asserts that Bryan's GFP, in combination with Aran's retroviral vectors, renders the subject matter of the instant claims obvious.

In view of the generally discussion set forth above, the Applicants submit that this rejection has been adequately addressed. Withdrawal of this rejection is respectfully requested.

**Rejection under 35 U.S.C. § 103 - Aran, Bryan and Zolutukhin**

Claim 20 is rejected under 35 U.S.C. § 103(a) as being unpatentable over Aran, Bryan and Zolutukhin. The Office Asserts that Aran's retroviral vectors, Bryan's Renilla GFP and Zolutukhin's human codon optimized GFP renders the subject matter of the instant claims obvious.

In view of the generally discussion set forth above, the Applicants submit that this rejection has been adequately addressed. Withdrawal of this rejection is respectfully requested.

**Rejection under 35 U.S.C. § 103 - Zolutukhin and Bryan**

Claims 1-3 and 20 are rejected under 35 U.S.C. § 103(a) as being unpatentable over Zolutukhin and Bryan. The Office Asserts that Zolutukhin's human codon optimized GFP retroviral vector, in combination with Bryan's *Renilla* GFP, renders the subject matter of the instant claims obvious.

In view of the generally discussion set forth above, the Applicants submit that this rejection has been adequately addressed. Withdrawal of this rejection is respectfully requested.

## CONCLUSION

The Applicants submits that all of the claims are in condition for allowance, which action is requested. If the Examiner finds that a telephone conference would expedite the prosecution of this application, please telephone the undersigned at the number provided.

The Commissioner is hereby authorized to charge any underpayment of fees associated with this communication, including any necessary fees for extensions of time, or credit any overpayment to Deposit Account No. 50-0815, order number RIGL-011.

Respectfully submitted,
BOZICEVIC, FIELD & FRANCIS LLP

Date: Feb 17, 2006

By: James S Keddie
James S. Keddie, Ph.D.
Registration No. 48,920

BOZICEVIC, FIELD & FRANCIS LLP
1900 University Avenue, Suite 200
East Palo Alto, CA 94303
Telephone: (650) 327-3400
Facsimile: (650) 327-3231

F:\DOCUMENT\RIGL (Rigel)\011\Response to OA dated August 18, 2005.doc